UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

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DEC 12 1989

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Tebuthiuron

Project No. 9-1734
TOX Chem No. 366AA

FROM: Ray Landolt 12/6/89
Review Section I
Toxicology Branch II - Herbicide, Fungicide, and
Antimicrobial Support
Health Effects Division (H7509C)

TO: Robert J. Taylor, PM 25
Fungicide-Herbicide Branch
Registration Division (H7505C)

THRU: Mike Ioannou, Section Head M. Ioannou 12/6/89
Review Section I
Toxicology Branch II - Herbicide, Fungicide, and
Antimicrobial Support
Health Effects Division (H7509C)

and

Marcia van Gemert, Branch Chief M van Gemert 12/7/89
Toxicology Branch II - Herbicide, Fungicide, and
Antimicrobial Support
Health Effects Division (H7509C)

Registrant: Elanco Products Company, letter of June 14, 1989

Action Requested

In response to the deficiencies noted in the Toxicology Chapter (July 1987) of the Registration Standard for Tebuthiuron,

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the registrant has submitted the following mutagenicity study to satisfy data requirement 84-2.

The Effect of Tebuthiuron (EL-103, Compound 075503) on the In Vitro Induction of Chromosomal Aberrations in Chinese Hamster Ovary Cells (MRID No. 411341-01).

Conclusion:

Classification of data - Acceptable.

Positive clastogenic effects at the highest levels assayed, 1950 ug/mL without S9 activation, and at 1550 ug/mL with S9 activation.

TOX Chem No. 366AA File Last Updated _____ Current Date _____

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	Toxicity Category	CORE Grade/ Doc. No.
Mutgenic-In vitro Cytogenic Species - CHO Cells; Elanco No. 890228CAB655; April 12, 1989	Tebuthiuron 99.08%	411341-01	Positive clastogenic effects with and without S9-activation at the highest dose assayed. Levels tested: 1650, 1800, and 1950 ug/mL without S9 activation; 1350, 1450, and 1550 ug/mL with S9 activation.		Acceptable

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EPA No.: 68D80056
DYNAMAC No.: 229-B
TASK No.: 2-29B
November 27, 1989

CONFIDENTIAL BUSINESS INFORMATION
DOES NOT CONTAIN
NATIONAL SECURITY INFORMATION (EO 12065)

DATA EVALUATION RECORD

TEBUTHIURON

Mutagenicity--In vitro Cytogenetic Study in Chinese Hamster Ovary Cells

APPROVED BY:

Robert J. Weir, Ph.D.
Program Manager
Dynamac Corporation

Signature: Robert J. Weir

Date: NOV 27 1989

EPA No.: 68D80056
DYNAMAC No.: 229-B
TASK No.: 2-29B
November 27, 1989

DATA EVALUATION RECORD

TEBUTHIURON

Mutagenicity--*In vitro* Cytogenetic Study in Chinese Hamster Ovary Cells

REVIEWED BY:

Nancy E. McCarroll, B.S.
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Signature: Nancy E. McCarroll
Date: 11-27-89

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Signature: M. Ioannou
Date: 12/6/89

DATA EVALUATION RECORD

CHEMICAL: Tebuthiuron.

STUDY TYPE: Mutagenicity--In vitro cytogenetic study in Chinese hamster ovary cells.

MRID NUMBER: 411341-01.

TEST MATERIAL: Tebuthiuron.

SYNONYMS/CAS No.: N-[5-(1,1-dimethylethyl)-1,3,4-thiadiazol-2-yl]-N,N'-dimethylurea; EL-103.

SPONSOR: Eli Lilly and Company, Greenfield, IN.

TESTING FACILITY: Lilly Research Laboratories, Greenfield, IN.

TITLE OF REPORT: The Effect of Tebuthiuron (EL-103, Compound 075503) on the In Vitro Induction of Chromosomal Aberrations in Chinese Hamster Ovary Cells.

AUTHOR(S): Negilski, D.S., Garriott, M.L., and Kindig, D.E.F.

STUDY NUMBER(S): 890111CTX655, 890125CTX655; 890201CAB655, and 890228CAB655 (note: different phases of this study were assigned different study numbers).

REPORT ISSUED: April 12, 1989.

CONCLUSIONS/EXECUTIVE SUMMARY:

Tebuthiuron was evaluated under nonactivated and S9-activated conditions in two independent assays for the potential to induce chromosome aberrations in Chinese hamster ovary (CHO) cells. Cultures exposed to 1650, 1800, and 1950 µg/mL without S9 activation and 1350, 1450, and 1550 µg/mL with S9 activation were evaluated for chromosome aberrations. Results indicated that only the highest nonactivated and S9-activated doses induced significant ($p<0.01$) and reproducible increases in the percentage of cells with aberrations. Under both nonactivated and S9-activated conditions, chromatid and chromosome breaks were the most frequently observed aberrations.

Although significant clastogenic effects occurred only at the highest assayed doses, the consistency of these findings together with the induction of rare complex aberrations at lower doses provided sufficient evidence to conclude that tebuthiuron is clastogenic in this in vitro test system.

Study Classification: The study is acceptable.

A. MATERIALS:

1. Test Material:
   Name: Tebuthiuron (EL-103, compound 075503).
   Description: The physical appearance of the test material was not reported; however, the chemical name was provided.
   Lot No.: 729AS7.
   Purity: 99.08%.
   Contaminants: None listed.
   Solvent Used: Dimethylsulfoxide (DMSO).
   Other Comments: The test material was stored at room temperature; solutions of the test material that were used in the different phases of this study were prepared on the day of use.

2. Cell Line: The Chinese hamster ovary (CHO) cells (subline WB_L) were originally obtained from Hazleton Laboratories America, Inc. Frozen stock cultures were maintained in liquid nitrogen. Working cultures, derived from the frozen stocks, were grown for 24 hours in McCoy's 5A medium supplemented with 10% fetal calf serum, L-glutamine, and antibiotics.

3. S9 Fraction: The S9 fraction was derived from the livers of male Fischer 344 rats induced with Aroclor 1254. The S9 reaction mixture contained 25% rat liver S9, 15 mg/mL isocitric acid and 8 mg/mL nicotinamide adenine dinucleotide phosphate.

4. Positive Control Compounds: The positive control compounds used in this assay were 0.5 µg/mL mitomycin C (MMC) for the nonactivated phase of testing and 5 µg/mL cyclophosphamide (CP) for the S9-activated phase of testing.

B. STUDY DESIGN:

1. Preliminary Cytotoxicity Assay: Prepared cultures, seeded at 1 x 10^5 cells/flask, were initially exposed with or without S9 activation to nine doses of the test material (1 to 1000 µg/mL) or the solvent control (DMSO). Following the 4-hour exposure, cells were washed, refed with complete medium, and reincubated for 16 to 18 hours. Cells were harvested by mitotic shake-off and counted; percent survival was determined by trypan blue exclusion. Based on these preliminary results, doses expected to yield 40 to 60% culture survival were selected for the cytogenetic assay.

2. Cytogenetic Assay:

a. Treatment: Prepared cultures (in triplicate) were exposed for 4 hours to the selected doses of the test material, solvent (DMSO), or positive controls (0.5 µg/mL MMC/-S9 or 5.0 µg/mL CP/+S9). Cells were washed, refed complete medium, and reincubated; 2 hours prior to cell harvest (19 hours postdosing), 0.1 µg/mL colcemid was added to two of the three replicate cultures to arrest cells in metaphase. Cultures containing nonarrested cells were used to assess cytotoxicity. Metaphase cells were collected, swollen with 0.075 M KCl, and fixed with methanol:glacial acetic acid (3:1). Slides were stained with 4% Giemsa and coded prior to scoring.

b. Metaphase Analysis: Fifty metaphase cells per culture in the solvent and treatment groups were scored for chromosome aberrations. Twenty-five cells each were scored from the nonactivated and S9-activated positive control groups. The number of aberrations per cell, percentage of cells with aberrations (including and excluding gaps), and the percentage of cells with more than one aberration were calculated.

3. Statistical Methods: The data were evaluated for statistical significance by the trend test for Poisson distribution described by Margolin et al.[1]

4. Evaluation Criteria:

   a. Assay Validity: The assay was considered valid if: 1) the chromosome aberration frequency in solvent and positive control groups was within the reporting laboratory's historical range; and 2) the test material was assayed to a cytotoxic level, the limit of solubility, or the maximum treatment level (10 mM).

   b. Positive Response: The test material was considered positive if it caused a significant and dose-related increase in chromosome aberrations relative to the solvent control.

C. REPORTED RESULTS:

1. Preliminary Cytotoxicity Assay: None of the doses evaluated in the initial cytotoxicity assay (1, 10, 50, 100, 200, 300, 500, 750, and 1000 µg/mL) were cytotoxic either in the presence or absence of S9 activation. Accordingly, the assay was repeated with six nonactivated and six S9-activated doses ranging from 1000 to 2285 µg/mL. In the nonactivated test, percent survival ranged from 115% at 1000 µg/mL to 15% at 2285 µg/mL; 55% survival was reported at 2000 µg/mL. Based on these findings, doses of 1500, 1650, 1800, 1950, and 2100 µg/mL were selected for the nonactivated cytogenetic assay. In the presence of S9 activation, ≤39% of the cells survived treatment with doses ≤1750 µg/mL; 55% cell survival was observed at 1500 µg/mL and no cytotoxicity was apparent at lower doses. Test concentrations of 1350, 1400, 1450, 1500, and 1550 µg/mL were, therefore, selected for the S9-activated cytogenetic assay.

2. Cytogenetic Assay: Two independent cytogenetic assays were performed with the test material. The results for each assay are discussed separately.

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[1]Margolin, B. H., Resnick, M. A., Pimpo, J. Y., Archer, P., Galloway, S. M., Bloom, A. D., and Zeiger, E. Statistical analysis for *in vitro* cytogenetic assays using Chinese hamster ovary cells. *Environmental Mutagen* 8(1986): 18-204.

a. Initial Assay: Based on the findings of the concurrent cytotoxicity test, three nonactivated (1650, 1800, and 1550 µg/mL) and three S9-activated (1350, 1450, and 1550 µg/mL) doses were scored for chromosome aberrations. As shown in Table 1, the highest nonactivated (1950 µg/mL) and the highest S9-activated (1550 µg/mL) doses induced significant ($p<0.01$) increases in the percentage of cells with aberrations. Under both conditions, the predominant types of induced aberrations were chromatid and chromosome breaks. No significant increases were seen at lower nonactivated or S9-activated doses; however, rare complex aberrations (e.g., triradials, quadriradials, and complex rearrangements) were scored at these levels. The presence of these rare aberrations provided further support that the test material was clastogenic.

b. Confirmation Assay: The independent repeat assay was conducted with similar nonactivated and S9-activated concentrations of tebuthiuron. Representative data presented in Table 2 indicated that the findings of the repeat assay were in good agreement with the results of the initial assay.

Based on reproducible evidence of significantly ($p<0.01$) increased chromosome aberration frequencies in cultures exposed to 1950 µg/mL/-S9 and 1550 µg/mL/+S9, the study authors concluded that tebuthiuron was clastogenic in this test system.

D. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

We assess that the study was properly conducted and that the study authors interpreted the data correctly. Tebuthiuron induced a significant increase in chromosome aberrations under nonactivated and S9-activated conditions; these findings were confirmed in an independent repeat assay. Additionally, the presence of rare complex aberrations at lower doses supports the conclusion of a positive clastogenic response.

We conclude, therefore, that tebuthiuron was clastogenic and that S9 activation was not required to demonstrate this effect.

E. QUALITY ASSURANCE: A quality assurance statement was signed and dated May 9, 1989.

F. CBI APPENDIX: Appendix A, Materials and Methods, CBI pp. 9-14; Appendix B, Protocol, CBI pp. 38-45.

TABLE 1. Representative Results of the Initial CHO Cell *in vitro* Cytogenetic Assay with Tebuthiuron

Substance	Dose (µg/mL)	S9 Activation	No. of Cells Scored	Relative Percent Survival	Aberrations per Cell[a]	% Cells with Aberrations[a]	% Cells with >1 Aberration	Biologically Significant Aberrations[b] (No./Type)
Solvent Control								
Dimethylsulfoxide	--	-	100	100	0.07	5	2	1TB; 5SB; 1D
		+	100	100	0.08	6	1	2TB; 6SB
Positive Control								
Mitomycin C	0.5	-	25	NR[c]	2.40	88*	56	34TB; 6TR; 2QR; 1CR; 2ID; 11SB; 1R; 2CI
Cyclophosphamide	5.0	+	25	NR	0.88	44*	12	8TB; 3TR; 2QR; 1CR; 1ID; 7SB
Test material								
Tebuthiuron	1650	-	100	100	0.10	4	2	5TB; 1TR; 2QR; 1CR; 1SB
	1800	-	100	83	0.11	7	2	5TB; 5SB; 1DM
	1950	-	100	57	0.32	15*	7	11TB; 1TR; 3QR; 1CR; 1ID; 14SB; 1CI
	1350	+	100	100	0.08	8	0	2TB; 6SB
	1450	+	100	77	0.06	6	0	1TB; 1TR; 4SB
	1550	+	100	58	0.33	18*	10	13TB; 4TR; 1QR; 1ID; 12SB; 1CI; 1DM

[a]Excluding gaps.

[b]Abbreviations used:
TB - Chromatid break
SB - Chromosome break
D - Dicentric
CR - Complex rearrangement
TR - Triradial
QR - Quadriradial
ID - Interstitial deletion
CI - Chromosome interchange
R - Ring
DM - "Double minute"

[c]NR = Not reported.

*Significantly higher than the solvent control ($p<0.01$) by trend analysis for Poisson distribution.

TABLE 2. Representative Results of the Confirmation CHO Cell *in vitro* Cytogenetic Assay with Tebuthiuron

Substance	Dose (µg/ml)	S9 Activation	No. of Cells Scored	Aberrations per Cell[a]	% Cells with Aberrations[a]	% Cells with >1 Aberration	Biologically Significant Aberrations[b] (No./Type)
Solvent Control							
Dimethylsulfoxide	--	-	100	0.05	5	0	3TB; [illegible]
		+	100	0.06	5	1	4TB
Positive Control							
Mitomycin C	0.5	-	25	0.92	60*	28	11TB; 2TR; 1QR 6SB; 1CI
Cyclophosphamide	5.0	+	25	0.72	40*	24	6TB; 1TR; 1CR; [illegible]; 1DM
Test Material							
Tebuthiuron	1650	-	100	0.13	8	3	7TB; 6SB
	1800	-	100	0.03	3	0	2TB; 1SB
	1950	-	100	0.35	19*	7	16TB; 3TR; 3QR; 2CR; 2ID; 7SB; 1CI; 1DM
	1350	+	100	0.08	8	0	4TB; 4SB
	1450	+	100	0.14	10	2	7TB; 3TR; 1QR; 1CR; 2SB
	1550	+	100	0.27	15*	6	5TB; 2QR; 2CR; 3ID; 11SB; 1R; 2CI; 1DM

[a]Excluding gaps.

[b]Abbreviations used:

TB - Chromatid break
SB - Chromosome break
D - Dicentric
CR - Complex rearrangement
TR - Triradial
QR - Quadriradial
ID - Interstitial deletion
CI - Chromosome intrachange
R - Ring
DM - "Double minute"

*Significantly higher than the solvent control ($p<0.01$) by trend analysis for Poisson distribution.

APPENDIX A

Materials and Methods
CBI pp. 9-14

Tebuthiuron Science Reviews

Page _____ is not included in this copy.

Pages 14 through 28 are not included in this copy.

The material not included contains the following type of information:

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